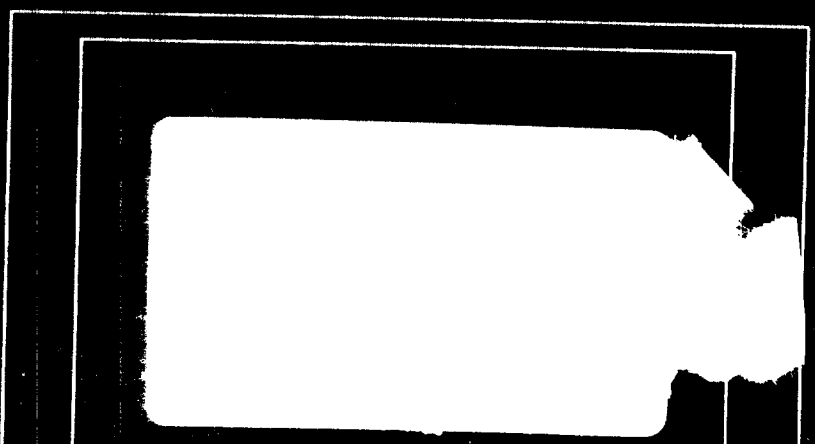
MUTAGENIC EVALUATION OF COMPOUND
FDA 71-62 COPPER GLUCONATE

Copper Salts Used in Food
or Gluconate

Litton

BIONETICS



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LBI PROJECT #2468

MUTAGENIC EVALUATION OF COMPOUND FDA 71-62 COPPER GLUCONATE

SUBMITTED TO

FOOD & DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
ROCKVILLE, MARYLAND

SUBMITTED BY

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Litton BIONETICS

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DATE: January 10, 1975

SPONSOR: Food and Drug Administration

SUBJECT: Mutagenic Evaluation of Compound FDA 71-62

I. OBJECTIVE

The objective of this study was to assess the genetic activity of the test material in microbial assays with and without the addition of mammalian metabolic enzyme preparations.

II. MATERIALS

A. Test Material

Copper Gluconate
Pfizer No. 72272

B. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from liver, lung and testes of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and primate - Macaca mulatta adult males.

C. Indicator Organisms

The indicator organisms used for all tests are described below:

- Saccharomyces cerevisiae, strain D4: $\frac{\alpha}{a}$, $\frac{\text{ade 2-2}}{\text{ade 2-1}}$, $\frac{\text{try 5-12}}{\text{try 5-27}}$

- Salmonella typhimurium, strains:

  TA-1535; hisG, uvrB, rfa (missense mutation)
  TA-1537; hisC, uvrB, rfa (- frameshift mutation)
  TA-1538; hisD, uvrB, rfa (+ frameshift mutation)

D. Reaction Mixture

The following reaction mixture was employed in the activation tests:

	Component	Final Concentration/ml
1.	TPN (sodium salt)	6 µM
2.	Isocitric acid	49 µM
3.	Tris buffer, pH 7.4	28 µM
4.	$MgCl_2$	1.7 µM
5.	Isocitric dehydrogenase	1.0 Unit
6.	Tissue homogenate or cell fraction	72 mg

Components 1-4 were combined and frozen as a "core" reaction mixture to which the other components were added just prior to use.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

ASSAY	CHEMICAL[a]	SOLVENT	PROBABLE MUTAGENIC SPECIFICITY[b]
Non-activation	Ethylmethane sulfonate	Water or saline	BPS
	2-Nitrosofluorene	Dimethylsulfoxide[c]	FS
	Quinacrine or Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS

[a] Concentrations given in the Results Section.

[b] BPS = base-pair substitution; FS = frameshift.

[c] Previously shown to be non-mutagenic, see Appendix.

III. METHODS

A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against strains TA-1537 and D4 over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival dose was determined from the survival curve and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests

Only three bacteria strains were tested in qualitative plate tests. In the non-activation procedure, approximately 10^9 cells of a log phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (Test, Positive Control and Solvent Control) was done in duplicate. The results were scored as + or -. Concentrations of the positive control compounds are listed in the Results Section.

C. Suspension Tests

1. Non-activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^9 cells/ml and 5×10^7 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in 30 ml plastic tissue culture flasks. Cells plus appropriate volume(s) of the test chemical were added to the flasks to give a final volume of 2 ml. Solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the flasks were set in ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the non-activation tests except that the cell densities were increased approximately five-fold for working stock suspensions. Measured amounts of the test and

control chemicals plus 0.25 ml of the stock cell suspension were added to a 30 ml plastic tissue homogenate. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non-activation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

1. Mice

Male mice (sufficient to provide the necessary quantities of organs for testes, lung and liver homogenates) were killed by cranial blow, decapitated and bled. The three organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

2. Rats

The same procedures as described for mice were used for this mammal.

3. Primates

The liver, lungs and testes were aseptically removed from freshly killed adult male rhesus (M. mulatta) monkeys. Each organ was cut into a number of pieces each sufficient for one week's studies. The tissues were labeled and frozen at -80°C until needed. Tissue homogenates and 9,000 x g supernatants were prepared as described for mice.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, in bound data books. Information necessary for identification of the specific experiment as well as the presence of any contaminant micro-organisms was recorded with each set of plate counts. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated.

Frequencies were mechanically calculated and double checked. All data presented in the Results Section of this report consists of the actual sum of all raw data plate counts and only the frequencies are calculated figures.

IV. SOLUBILITY PROPERTIES OF THE TEST COMPOUND

1. NAME OR DESCRIPTION OF TEST COMPOUND:

   Copper Gluconate

2. TEST SOLVENT AND DESCRIPTION OF SOLUBILITY OF THE TEST CHEMICAL UNDER TREATMENT CONDITIONS:

   This compound was soluble at the treatment concentration employed in this evaluation. All tests were conducted in an aqueous environment.

3. OTHER COMMENTS:

V. TOXICITY AND DOSAGE DETERMINATIONS COMPOUND FDA 71-62

		D4	TA-1537
	Dose No.	% Concentration	% Concentration
Range of concentrations of the test compound used to determine the 50% survival level	1	0.01	0.00001
	2	0.05	0.00005
	3	0.1	0.0001
	4	0.25	0.0005
	5	0.5	0.001
	Dose No.	% Survival	% Survival
Survival Results	Control	100	100
	1	97	100
Test Date: _____	2	37	100
	3	22	100
	4	3	80
	5	1	5
	Dose	% Concentration	% Concentration
Concentrations of the test chemical required for mutagenicity tests	Plate Test	----	0.0035
	¼ 50% Survival	0.008	0.0017
	½ 50% Survival	0.017	0.0035
	Other		

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VI. NON-ACTIVATION PLATE TESTS

DATE: 11-20-74

Test	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	EMS	0.05 ml undiluted chemical	$>10^3$	$>10^3$				
	QM	0.25 mg			$>10^2$	$>10^2$		
	NF	0.25 mg					$>10^2$	$>10^2$
SC	SALINE	-	2	1	2	4		
	DMSO	<10%					5	1

NOTE: PC = positive control
SC = solvent control
T-1 = trial 1
T-2 = trial 2
EMS = ethyl methanesulfonate
QM = quinacrine mustard
NF = nitrosofluorene
DMSO = dimethyl sulfoxide
(c) = contamination present

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EVALUATION SUMMARY

Compound FDA 71-62, Copper Gluconate, did not exhibit genetic activity in any of the *in vitro* tests employed in this evaluation.

NON-ACTIVATION PLATE TESTS

DATE: 11-20-74

Test	Compound	Concentration	TA-1535		TA-1537		TA-1538	
			T-1	T-2	T-1	T-2	T-1	T-2
TC	FDA 71-62	0.00315%	2	2	1	3	6	4

NOTE: TC = test compound
T-1 = trial 1
T-2 = trial 2
(c) = contamination present

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VII. ACTIVATION PLATE TESTS

SPECIES: MOUSE DATE: 11-20-74

Test	Organ	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	Li	DMNA	25 µmoles	$>10^3$	$>10^3$				
		AAF	1.25 mg			44	43	$>10^2$	$>10^2$
	Lu	DMNA	25 µmoles	2	4				
		AAF	1.25 mg			9	3	13	8
	T	DMNA	25 µmoles	1	5				
		AAF	1.25 mg			6	10	3	3
SC	-	DMNA	25 µmoles	3	0				
	-	AAF	1.25 mg			10	5	1	0
	-	Saline	-	1	1				
	-	DMSO	<10%			12	10	6	7

NOTE:
PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
DMSO = dimethyl sulfoxide
(c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: MOUSE DATE: 11-20-74

Test	Organ	Compound	Concentration	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
TC	Li	FDA 71-62	0.00315%	0	0	23	24	7	0
	Lu	FDA 71-62	0.00315%	1	2	5	9	9	13
	T	FDA 71-62	0.00315%	2	0	25	20	16	14

NOTE: TC = test compound
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
(c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: RAT

DATE: 11-20-74

Test	Organ	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	Li	DMNA	25 µmoles	$>10^2$	$>10^2$				
		AAF	1.25 mg			41	30	$>10^2$	$>10^2$
	Lu	DMNA	25 µmoles	1	0				
		AAF	1.25 mg			7	10	5	0
	T	DMNA	25 µmoles	3	0				
		AAF	1.25 mg			14	17	10	3
SC	-	DMNA	25 µmoles	3	0				
	-	AAF	1.25 mg			10	5	1	0
	-	Saline	-	1	1				
	-	DMSO	<10%			12	10	6	7

NOTE:
PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
DMSO = dimethyl sulfoxide
(c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: RAT DATE: 11-20-74

Test	Organ	Compound	Concentration	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
TC	Li	FDA 71-62	0.00315%	2	4	11	8	7	12
	Lu	FDA 71-62	0.00315%	1	2	6	10	4	9
	T	FDA 71-62	0.00315%	3	2	9	5	10	8

NOTE: TC = test compound
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
(c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: MONKEY

DATE: 11-20-74

Test	Organ	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	Li	DMNA	25 µmoles	$>10^2$	$>10^2$				
		AAF	1.25 mg			32	46	$>10^2$	$>10^2$
	Lu	DMNA	25 µmoles	0	4				
		AAF	1.25 mg			20	13	2	4
	T	DMNA	25 µmoles	1	1				
		AAF	1.25 mg			10	11	3	7
SC	-	DMNA	25 µmoles	3	0				
	-	AAF	1.25 mg			10	5	1	0
	-	Saline	-	1	1				
	-	DMSO	<10%			12	10	6	7

NOTE:
PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
DMSO = dimethyl sulfoxide
(c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: MONKEY DATE: 11-20-74

Test	Organ	Compound	Concentration	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
TC	Li	FDA 71-62	0.00315%	1	0	21	18	11	11
	Lu	FDA 71-62	0.00315%	0	0	16	30	7	14
	T	FDA 71-62	0.00315%	2	2	20	17	8	4

NOTE: TC = test compound
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
(c) = contamination present

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VIII. NON-ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

DATE: 10-9-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
PC	TA-1535	EMS	0.05 %	6.33	6990	1104.27
	TA-1537	QM	0.01 mg/ml	4.05	469	115.80
	TA-1538	NF	1.25 mg/ml	4.92	241	48.98
SC	TA-1535	SALINE	-	5.47	8	1.46
	TA-1537	SALINE	-	4.32	51	11.81
	TA-1538	DMSO	-	5.09	54	10.61

NOTE:
PC = positive control
SC = solvent control
EMS = ethyl methanesulfonate
QM = quinacrine mustard
NF = nitrosofluorene
DMSO = dimethyl sulfoxide
(c) = contamination present

NON-ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

DATE: 10-9-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ mlx10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
TC	TA-1535	FDA 71-62	H	6.81(124)	13(c)	1.91
TC	TA-1535	FDA 71-62	L	4.47(82)	6	1.34
TC	TA-1537	FDA 71-62	H	6.36(147)	52	8.18
TC	TA-1537	FDA 71-62	L	3.41(80)	58	7.01
TC	TA-1538	FDA 71-62	H	2.59(51)	53	20.46
TC	TA-1538	FDA 71-62	L	7.69(151)	63	8.19

NOTE: TC = test compound
H = high dose
L = low dose
(c) = contamination present
( ) = percent survival

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IX. ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MOUSE

DATE: 10-7-74					Strain TA-1535	
Test	Organ	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
PC	Li	DMNA	100 µmoles/ml	3.00	2195	731.67
	Lu	DMNA	100 µmoles/ml	1.67	11	6.59
	T	DMNA	100 µmoles/ml	1.78	10	5.62
SC	-	DMNA	100 µmoles/ml	5.41	12	2.22
	-	SALINE	-	4.54	11(c)	2.42

DATE: 10-8-74					Strain TA-1537	
Test	Organ	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml	5.63	85	15.10
	Lu	AAF	1.25 mg/ml	5.86	29	4.95
	T	AAF	1.25 mg/ml	5.53	12	2.17
SC	-	AAF	1.25 mg/ml	4.24	36	8.49
	-	DMSO	-	5.74	38	6.62

DATE: 10-9-74					Strain TA-1538	
Test	Organ	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml	8.58	256	29.84
	Lu	AAF	1.25 mg/ml	7.74	55	7.11
	T	AAF	1.25 mg/ml	6.17	53	8.59
SC	-	AAF	1.25 mg/ml	6.79	48	7.07
	-	DMSO	-	7.90	46	5.82

NOTE: PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
DMSO = dimethyl sulfoxide
(c) = contamination present

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ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

SPECIES: MOUSE

Test	Organ	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
DATE: 10-7-74				Strain TA-1535		
TC	Li	FDA 71-62	H	2.92(64)	4	1.37
		FDA 71-62	L	1.88(41)	8	4.26
	Lu	FDA 71-62	H	5.85(129)	1	0.17
		FDA 71-62	L	2.62(58)	2	0.76
	T	FDA 71-62	H	4.02(89)	8	2.00
		FDA 71-62	L	3.41(75)	5	1.47
DATE: 10-8-74				Strain TA-1537		
TC	Li	FDA 71-62	H	3.21(56)	56	7.45
		FDA 71-62	L	2.88(50)	28	9.72
	Lu	FDA 71-62	H	3.39(59)	25	7.38
		FDA 71-62	L	3.79(66)	30	7.92
	T	FDA 71-62	H	4.42(77)	36	8.15
		FDA 71-62	L	4.51(79)	36	7.98
DATE: 10-9-74				Strain TA-1538		
TC	Li	FDA 71-62	H	6.29(80)	47	7.47
		FDA 71-62	L	4.52(57)	44	9.74
	Lu	FDA 71-62	H	2.81(36)(c)	47	16.73
		FDA 71-62	L	4.25(54)	17	4.00
	T	FDA 71-62	H	8.02(102)	46	5.74
		FDA 71-62	L	4.09(52)	25	6.11

NOTES: H = high dose
L = low dose
TC = test compound
Li = liver
Lu = lung
T = testes
(c) = contamination present
( ) = percent survival

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ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: RAT

DATE: 10-11-74				Strain TA-1535		
Test	Organ	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
PC	Li	DMNA	100 µmoles/ml	8.06	1980	245.70
	Lu	DMNA	100 µmoles/ml	15.41	15	0.97
	T	DMNA	100 µmoles/ml	7.72	13	1.68
SC	-	DMNA	100 µmoles/ml	9.31	31	3.33
	-	SALINE	-	11.22	30	2.67

DATE: 10-31-74				Strain TA-1537		
Test	Organ	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml	3.13	92	29.39
	Lu	AAF	1.25 mg/ml	5.31	54	10.17
	T	AAF	1.25 mg/ml	2.83	25	8.83
SC	-	AAF	1.25 mg/ml	2.34	40	17.09
	-	DMSO	-	3.99	30	7.52

DATE: 10-23-74				Strain TA-1538		
Test	Organ	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml	2.29	98	42.80
	Lu	AAF	1.25 mg/ml	7.97	47	5.90
	T	AAF	1.25 mg/ml	6.41	39	6.08
SC	-	AAF	1.25 mg/ml	9.02	35	3.88
	-	DMSO	-	5.97	57	9.55

NOTE: PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
DMSO = dimethyl sulfoxide

(c) = contamination present

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ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

SPECIES: RAT

Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
DATE: 10-11-74				Strain TA-1535		
TC	Li	FDA 71-62	H	7.69(69)	19	2.47
		FDA 71-62	L	5.75(51)	21	3.65
	Lu	FDA 71-62	H	8.22(73)	20	2.43
		FDA 71-62	L	19.13(170)	17	0.89
	T	FDA 71-62	H	9.17(82)	15	1.64
		FDA 71-62	L	12.74(114)	17	1.33
DATE: 10-31-74				Strain TA-1537		
TC	Li	FDA 71-62	H	4.43(111)(c)	40	9.03
		FDA 71-62	L	2.48(62)	30	12.10
	Lu	FDA 71-62	H	4.46(112)	44	9.87
		FDA 71-62	L	4.59(115)	42	9.15
	T	FDA 71-62	H	1.80(45)	18(c)	10.00
		FDA 71-62	L	1.36(34)	14	10.29
DATE: 10-23-74				Strain TA-1538		
TC	Li	FDA 71-62	H	3.28(55)	25	7.62
		FDA 71-62	L	5.97(100)	22	3.69
	Lu	FDA 71-62	H	3.26(55)	15	4.60
		FDA 71-62	L	--------	37	-----
	T	FDA 71-62	H	3.23(54)	16	4.95
		FDA 71-62	L	3.13(52)	15	4.79

NOTES: H = high dose
L = low dose
TC = test compound
Li = liver
Lu = lung
T = testes
(c) = contamination present
( ) = percent survival

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ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MONKEY

DATE: 10-25-74 — Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	DMNA	100 µmoles/ml	5.24	1082	206.49
	Lu	DMNA	100 µmoles/ml	6.83	17	2.49
	T	DMNA	100 µmoles/ml	4.66	13	2.79
SC	-	DMNA	100 µmoles/ml	5.85	25	4.27
	-	SALINE	-	8.83	22	2.49

DATE: 10-24-74 — Strain TA-1537

Test	Organ	Compound	Concentration	Total Cells/ mlx10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml	3.78	101	26.72
	Lu	AAF	1.25 mg/ml	8.80	76	8.64
	T	AAF	1.25 mg/ml	8.68	60	6.91
SC	-	AAF	1.25 mg/ml	4.16	76	18.27
	-	DMSO	-	4.20	68	16.19

DATE: 10-30-74 — Strain TA-1538

Test	Organ	Compound	Concentration	Total Cells/ mlx10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml	2.21	67	30.32
	Lu	AAF	1.25 mg/ml	2.03	28	13.79
	T	AAF	1.25 mg/ml	2.64	23	8.71
SC	-	AAF	1.25 mg/ml	2.94	27	9.18
	-	DMSO	-	3.25	45	13.85

NOTE: PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
DMSO = dimethyl sulfoxide

(c) = contamination present

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

SPECIES: MONKEY

Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
DATE: 10-25-74				Strain TA-1535		
TC	Li	FDA 71-62	H	6.76(77)	15	2.22
		FDA 71-62	L	5.80(66)	15	2.59
	Lu	FDA 71-62	H	5.57(63)	7	1.25
		FDA 71-62	L	7.23(82)	15	2.08
	T	FDA 71-62	H	4.80(54)	12	2.50
		FDA 71-62	L	5.26(60)	13	2.47
DATE: 10-24-74				Strain TA-1537		
TC	Li	FDA 71-62	H	4.15(99)	73	17.59
		FDA 71-62	L	5.55(132)	86	15.50
	Lu	FDA 71-62	H	6.58(157)(c)	53	8.06
		FDA 71-62	L	5.84(139)	52	8.90
	T	FDA 71-62	H	5.01(119)	45(c)	8.82
		FDA 71-62	L	5.28(126)	56	10.61
DATE: 10-30-74				Strain TA-1538		
TC	Li	FDA 71-62	H	3.42(105)	41(c)	11.99
		FDA 71-62	L	2.95(91)(c)	32(c)	10.85
	Lu	FDA 71-62	H	3.25(100)	42	12.92
		FDA 71-62	L	2.45(75)	21(c)	8.57
	T	FDA 71-62	H	2.15(66)(c)	10	4.65
		FDA 71-62	L	3.56(110)	14	3.93

NOTES:
H = high dose
L = low dose
TC = test compound
Li = liver
Lu = lung
T = testes
(c) = contamination present
( ) = percent survival

Project No. 2468

X. NON-ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

DATE: 11-1-74

			Strain D4				
Test	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
PC	EMS	1.0 %	7.98	715	806	89.60	112.73
SC	Saline	-	9.62	63	42	6.55	4.37

NOTE: PC = positive control
SC = solvent control
EMS = ethyl methanesulfonate

a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present

Project No. 2468

NON-ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

DATE: 11-1-74

			Strain D4				
Test	Compound	Concentration	Total Population Screened[a]	Number Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
TC	FDA 71-62	H	8.00(83)	35	28	4.38	3.50
	FDA 71-62	L	7.06(73)	31	22	4.39	3.12

NOTE: TC = test compound
H = high dose
L = low dose
a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present
( ) = percent survival

Project No. 2468

XI. ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MOUSE DATE: 11-26-74

					Strain D4			
Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
PC	Li	DMNA	150 µmoles/ml	7.93	65	70	8.20	8.83
	Lu	DMNA	150 µmoles/ml	7.22	44	33	6.09	4.57
	T	DMNA	150 µmoles/ml	7.39	27	40	3.65	5.41
SC	-	DMNA	150 µmoles/ml	8.76	54	31(c)	6.16	3.54
	-	SALINE	-	8.66	48	40	5.54	4.62

NOTE: PC = positive control
SC = solvent and chemical controls
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes

a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: MOUSE

DATE: 11-26-74

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
TC	Li	FDA 71-62	H	(c)7.57(87)	41(c)	30(c)	5.42	3.96
		FDA 71-62	L	(c)9.83(114)	63	38	6.41	3.87
	Lu	FDA 71-62	H	9.21(106)	62	38	6.73	4.12
		FDA 71-62	L	7.98(92)	48	36	6.02	4.51
	T	FDA 71-62	H	8.06(93)	42(c)	33	5.21	4.09
		FDA 71-62	L	8.44(97)	40	43	4.74	5.09

NOTE:
TC = test compound
H = high dose
L = low dose
Li = liver
Lu = lung
T = testes
a = number x 10^5
b = number at 10^{-1} dilution
(c)= contamination present
( )= percent survival

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: RAT DATE: 10-25-74

					Strain D4			
Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
PC	Li	DMNA	150 µmoles/ml	5.84(c)	55	60	9.42	10.27
	Lu	DMNA	150 µmoles/ml	8.18	32	29	3.91	3.55
	T	DMNA	150 µmoles/ml	4.45	1	9	0.23	2.02
SC	-	DMNA	150 µmoles/ml	9.61	26	24	2.71	2.50
	-	SALINE	-	12.35	42	36	3.40	2.92

NOTE: PC = positive control
SC = solvent and chemical controls
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes

a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present

Project No. 2468

ACTIVATION SUSPENSION TESTS
WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: RAT

DATE: 10-25-74

				Strain D4				
Test	Organ	Compound	Concentration	Total Population Screened[d]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
TC	Li	FDA 71-62	H	8.53(69)	21	29	2.46	3.31
		FDA 71-62	L	10.78(87)	37	28	3.43	2.60
	Lu	FDA 71-62	H	8.58(69)	49	28	5.71	3.26
		FDA 71-62	L	10.29(83)	31	33	3.21	3.01
	T	FDA 71-62	H	8.32(67)	48	37(c)	5.77	4.45
		FDA 71-62	L	10.10(82)	21	36(c)	2.08	3.56

NOTE:
TC = test compound
H = high dose
L = low dose
Li = liver
Lu = lung
T = testes
a = number x 10^5
b = number at 10^{-1} dilution
(c)= contamination present
( )= percent survival

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MONKEY DATE: 11-7-74

					Strain D4			
Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
PC	Li	DMNA	150 µmoles/ml	7.44	73	21	9.81	2.82
	Lu	DMNA	150 µmoles/ml	9.39	37	34	3.94	3.62
	T	DMNA	150 µmoles/ml	7.04(c)	50	27	7.10	3.84
SC	-	DMNA	150 µmoles/ml	9.12(c)	37	13	4.06	1.43
	-	SALINE	-	9.79	28	13	2.86	1.33

NOTE: PC = positive control
SC = solvent and chemical controls
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes

a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: MONKEY DATE: 11-7-74

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
TC	Li	FDA 71-62	H	10.69(109)	39	35	3.65	3.27
		FDA 71-62	L	9.41(96)	36(c)	31	3.83	3.29
	Lu	FDA 71-62	H	10.06(103)	20	80	1.99	7.95
		FDA 71-62	L	8.04(82)	35	40	4.35	4.98
	T	FDA 71-62	H	9.95(102)	15	60	1.51	6.03
		FDA 71-62	L	8.80(90)	--	40	----	4.54

NOTE:
TC = test compound
H = high dose
L = low dose
Li = liver
Lu = lung
T = testes
a = number x 10^5
b = number at 10^{-1} dilution
(c)= contamination present
( )= percent survival

Project No. 2468

XII. SUMMARY OF TEST RESULTS

COMPOUND FDA 71-62

A. Suspension Tests

Test[a]	Activation Species[b]	Activation Organ[c]	Salmonella Reversion Frequencies (x 10^{-8}) TA-1535	TA-1537	TA-1538	Saccharomyces D4 Conversion Frequencies (x 10^{-5}) Ade^+	Try^+
NA-PC	-	-	1104.27	115.80	48.98	89.60	112.73
NA-NC	-	-	1.46	11.81	10.61	6.55	4.37
NA-H	-	-	1.91	8.18	20.46	4.38	3.50
NA-L	-	-	1.34	7.01	8.19	4.39	3.12
A-NC (-C)	-	-	2.42	6.62	5.82	5.54	4.62
A-NC (+C)	-	-	2.22	8.49	7.07	6.16	3.54
A-PC	M	Li	731.67	15.10	29.84	8.20	8.83
A-PC	M	Lu	6.59	4.95	7.11	6.20	4.57
A-PC	M	T	5.62	2.17	8.59	6.09	5.41
A-H	M	Li	1.37	7.45	7.47	5.42	3.96
A-L			4.26	9.72	9.74	6.41	3.87
A-H	M	Lu	0.17	7.38	16.73	6.73	4.12
A-L			0.76	7.92	4.00	6.02	4.51
A-H	M	T	2.00	8.15	5.74	5.21	4.09
A-L			1.47	7.98	6.11	4.74	5.09

[a] NA = non activation; NC = negative control; PC = positive control; A = activation; H = high dose; L = low dose

[b] M = mouse; Mo = monkey; R = rat

[c] Li = liver; Lu = lung; T = testes

(-C) = solvent control; (+C) = chemical control

Project 2468

COMPOUND FDA 71-62

B. Plate Tests

Test[a]	Activation		Salmonella Responses		
	Species[b]	Organ[c]	TA-1535	TA-1537	TA-1538
NA-PC	-	-	+	+	+
NA-NC	-	-	-	-	-
NA-H	-	-	-	-	-
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	M	Li	+	+	+
A-PC	M	Lu	-	-	-
A-PC	M	T	-	-	-
A-H	M	Li	-	-	-
A-H	M	Lu	-	-	-
A-H	M	T	-	-	-

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

Project 2468

SUMMARY OF TEST RESULTS

COMPOUND FDA 71-62

A. Suspension Tests

Test[a]	Activation Species[b]	Activation Organ[c]	Salmonella Reversion Frequencies (x 10^{-8}) TA-1535	TA-1537	TA-1538	Saccharomyces D4 Conversion Frequencies (x 10^{-5}) Ade^+	Try^+
NA-PC	-	-					
NA-NC	-	-					
NA-H	-	-					
NA-L	-	-					
A-NC (-C)	-	-	2.67	7.52	9.55	3.40	2.92
A-NC (-C)	-	-	3.33	17.09	3.88	2.71	2.50
A-PC	R	Li	245.70	29.39	42.80	9.42	10.27
A-PC	R	Lu	0.97	10.17	5.90	3.91	3.55
A-PC	R	T	1.68	8.83	6.08	0.23	2.02
A-H	R	Li	2.47	9.03	7.62	2.46	3.31
A-L			3.65	12.10	3.69	3.43	2.60
A-H	R	Lu	2.43	9.87	4.60	5.71	3.26
A-L			0.89	9.15	----	3.21	3.01
A-H	R	T	1.64	10.00	4.95	5.77	4.45
A-L			1.33	10.29	4.79	2.08	3.56

[a] NA = non activation; NC = negative control; PC = positive control; A = activation; H = high dose; L = low dose

[b] M = mouse; Mo = monkey; R = rat

[c] Li = liver; Lu = lung; T = testes

(-C) = solvent control
(+C) = chemical control

Project 2468

COMPOUND FDA 71-62

B. Plate Tests

Test[a]	Activation		Salmonella Responses		
	Species[b]	Organ[c]	TA-1535	TA-1537	TA-1538
NA-PC	-	-			
NA-NC	-	-			
NA-H	-	-			
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	R	Li	+	+	+
A-PC	R	Lu	-	-	-
A-PC	R	T	-	-	-
A-H	R	Li	-	-	-
A-H	R	Lu	-	-	-
A-H	R	T	-	-	-

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

Project 2468

SUMMARY OF TEST RESULTS

COMPOUND FDA 71-62

A. Suspension Tests

Test[a]	Activation Species[b]	Activation Organ[c]	Salmonella Reversion Frequencies (x 10^{-8}) TA-1535	TA-1537	TA-1538	Saccharomyces D4 Conversion Frequencies (x 10^{-5}) Ade^+	Try^+
NA-PC	-	-					
NA-NC	-	-					
NA-H	-	-					
NA-L	-	-					
A-NC (-C)	-	-	2.49	16.19	13.85	2.86	1.33
A-NC (+C)	-	-	4.27	18.27	9.18	4.06	1.43
A-PC	Mo	Li	206.49	26.72	30.32	9.81	2.82
A-PC	Mo	Lu	2.49	8.64	13.79	3.94	3.62
A-PC	Mo	T	2.79	6.91	8.71	7.10	3.84
A-H	Mo	Li	2.22	17.59	11.99	3.65	3.27
A-L			2.59	15.50	10.85	3.83	3.29
A-H	Mo	Lu	1.25	8.06	12.92	1.99	7.95
A-L			2.08	8.90	8.57	4.35	4.98
A-H	Mo	T	2.50	8.82	4.65	1.51	6.03
A-L			2.47	10.61	3.93	----	4.54

[a] NA = non activation; NC = negative control; PC = positive control; A = activation; H = high dose; L = low dose

[b] M = mouse; Mo = monkey; R = rat

[c] Li = liver; Lu = lung; T = testes

(-C) = solvent control; (+C) = chemical control

Project 2468

COMPOUND FDA 71-62

B. Plate Tests

Test[a]	Activation: Species[b]	Activation: Organ[c]	Salmonella Responses: TA-1535	TA-1537	TA-1538
NA-PC	-	-			
NA-NC	-	-			
NA-H	-	-			
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	Mo	Li	+	+	+
A-PC	Mo	Lu	-	-	-
A-PC	Mo	T	-	-	-
A-H	Mo	Li	-	-	-
A-H	Mo	Lu	-	-	-
A-H	Mo	T	-	-	-

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

Project 2468

XIII. INTERPRETATION AND CONCLUSIONS

Compound FDA 71-62, Copper Gluconate, was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. *Salmonella typhimurium*

1. Plate Tests

At a concentration of 0.00025%, this compound was not mutagenic for TA-1535, TA-1537 or TA-1538 in direct or activation plate tests.

2. Non-activation Suspension Tests

These tests were negative.

3. Activation Suspension Tests

These tests were negative.

B. *Saccharomyces cerevisiae*

1. Non-activation Suspension Tests

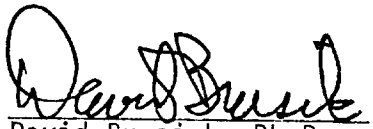
These tests were negative.

2. Activation Suspension Tests

These tests were negative.

C. Conclusions

Compound FDA 71-62, Copper Gluconate, was not genetically active for bacterial and yeast indicator organisms under the conditions of this evaluation.

SUBMITTED BY: 

David Brusick, Ph.D.
Director
Department of Genetics

APPENDIX

SUMMARY OF TESTS EVALUATING DMSO FOR GENETIC ACTIVITY IN *SALMONELLA* AND *SACCHAROMYCES*

Litton BIONETICS

COMPOUND DIMETHYSULFOXIDE

A. Suspension Tests

Test	Activation: Species[a]	Activation: Organ[b]	Salmonella Reversion Frequencies (x 10^{-8}): TA-1535	Salmonella Reversion Frequencies (x 10^{-8}): TA-1538	Saccharomyces D4 Conversion Frequencies (x 10^{-5}): Ade+	Saccharomyces D4 Conversion Frequencies (x 10^{-5}): Try+
Non-activation						
Control (-C)	-	-	0.74	0.88	32.51	4.34
High Dose[c]	-	-	1.91	1.05	28.32	2.95
Low Dose[d]	-	-	0.53	1.37	40.73	0.49
Activation						
Control (+C)	-	-	1.80	0.36	38.27	2.47
Control (-C)	-	-	1.43	1.04	37.12	2.64
High Dose[c]	M	Li	0.34	1.07	47.77	2.75
	M	Lu	0.59	0.58	36.29	1.39
	M	T	0.62	0.30	34.35	1.94
Lose Dose[d]	M	Li	-	0.87	34.02	1.18
	M	Lu	0.43	3.14	42.30	1.40
	M	T	0.11	0.39	45.95	2.32

Note: (-C) = solvent control and (+C) = test chemical control without homogenate

a M = mouse, Mo = monkey, R = rat

b Li = liver, Lu = lung, T = testes

c Bacteria = 3%, Yeast = 5%

d Bacteria = 1.5%, Yeast = 2.5%

Litton BIONETICS

COMPOUND DIMETHYSULFOXIDE

B. Plate Tests

Test	Activation Species[a]	Activation Organ[b]	Salmonella Responses TA-1535	TA-1537	TA-1538
Non-activation					
Control (-C)	-	-	-	-	-
Test compound (3%)	-	-	-	-	-
Activation					
Control (+C)	-	-	-	-	-
Control (-C)	-	-	-	-	-
Test compound (3%)	M	Li	-	-	-
	M	Lu	-	-	-
	M	T	-	-	-
	R	Li	-	-	-
	R	Lu	-	-	-
	R	T	-	-	-
	Mo	Li	-	-	-
	Mo	Lu	-	-	-
	Mo	T	-	-	-

Note: (-C) = solvent control and (+C) = chemical control without homogenate

a M = mouse
Mo = monkey
R = rat

b Li = liver
Lu = lung
T = testes

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

Glutamic Acid

TO : Mr. Alan Spiher
GRAS Review Branch, HFF-335

DATE: February 14, 1975

THRU : Dr. Herbert Blumenthal, Acting Director
Division of Toxicology, HFF-150

FROM : M. Jacqueline Verrett, Ph.D. M Jacqueline Verrett
Reproductive Physiology Branch, HFF-157

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Mono-Potassium Glutamate in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Mono Potassium Glutamate

Protocol:

Mono Potassium Glutamate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Colum 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Air cell treatment at 0 hours showed no toxicity above background. When administered via the air cell at 96 hours there was a regression of mortality on dose with a calculated LD_{50} of 306.7 mg/kg (10.8 mg/egg). Yolk treatment at both times resulted in a regression line whose slope was not significantly different from zero ($p=0.05$).

Scattered abnormalities were observed for all four conditions of test, but in no instances were these significantly higher than or different from those observed in the solvent-treated or untreated control eggs. Mono Potassium Glutamate displayed no teratogenicity under the test conditions employed.

1. Mono Potassium Glutamate, Lot # 022874B61, Stauffer Chemical Co., Westport, Conn.

2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.

3. Verrett, M.J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002 - 1006.

4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Table 1

Mono Potassium Glutamate

A.C. @ 0 Hours

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.00	200.00	105	22.85	6.66	2.85
5.00	100.00	105	22.85	2.85	0.95
2.50	50.00	105	28.57	3.80	0.95
1.250	25.00	105	20.00	7.61	0.00
0.500	10.00	105	25.71	8.57	3.80
Water		134	20.89	2.23	0.74
Controls		408	12.00	1.96	0.98

** Slope is negative

Table 2

Mono Potassium Glutamate

A.C. @ 96 Hours

Dose /egg	mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
.00	100.00	120	44.16*	1.66	1.66
.50	50.00	119	21.00	2.52	0.84
.250	25.00	118	22.88	4.23*	1.69
.6250	12.50	120	22.50	2.50	0.83
.250	5.00	120	19.16	2.50	1.66
ter		140	17.14	0.00	0.00
ntrols		408	12.00	1.96	0.98

** LD_{50} 306.7693 mg/kg (10.8384 mg/egg)

* Significantly different from solvent ($p \leq 0.05$)

Mono Potassium Glutamate

Yolk @ 0 Hours

Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
g/egg	mg/kg			Total	Structural
10.00	200.00	105	78.09*	1.90	1.90
5.00	100.00	105	65.71*	0.00	0.00
2.50	50.00	104	72.11*	1.92	0.00
1.250	25.00	105	67.61*	1.90	1.90
0.500	10.00	105	58.09*	2.85	1.90
Water		138	25.36	0.72	0.72
Controls		408	12.00	1.96	0.98

** Slope not significantly different from zero ($p=0.05$)

* Significantly different from solvent ($p \leq 0.05$)

Table 4

Mono Potassium Glutamate

Yolk @ 96 Hours

Dose µg/egg	Dose mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	105	57.14*	4.76	3.80
2.50	50.00	105	58.09*	2.85	0.95
1.250	25.00	105	56.19*	4.76	2.85
0.6250	12.50	105	48.57	7.61	2.85
0.250	5.00	105	53.33*	4.76	1.90
Water		115	36.52	3.47	2.60
Controls		408	12.00	1.96	0.98

** Slope not significantly different from zero (p=0.05)

* Significantly different from solvent ($p \leq 0.05$)